Department of Veterans Affairs

Journal of Rehabilitation Research and Development Vol. 37 No. 2, March/April 2000
Pages 171–177

In vitro engineering of cartilage

Julie Glowacki, PhD
Department of Orthopedic Surgery, Brigham and Women's Hospital, Harvard Medical School, and Skeletal Biology Research Center, Massachusetts General Hospital, Department of Oral and Maxillofacial Surgery, Harvard School of Dental Medicine

Abstract–Because adult human cartilage shows poor capacity for repair and regeneration, innovative solutions are required for congenital and acquired degenerative cartilage lesions. Acquired lesions occur in young and old alike, the former being more at risk for sports-related injuries and the latter for age-related degenerative changes. Because cartilage is a relatively simple tissue with respect to its cellular homogeneity and avascularity, it has been a model for research of *in vitro* engineered tissues. Progress has been slow and obstructed on several levels. The adult chondrocyte has limited capacity for proliferation and has both catabolic and anabolic functions. These metabolic features must be controlled in order for engineered tissue to endure. Use of three-dimensional scaffolds can be combined with regulatory factors (cytokine, extracellular matrix [ECM], and mechanical) to optimize conditions for *in vitro* engineered cartilage. Cross-disciplinary interactions are likely to accelerate progress and to mediate application of advances made in other fields for consistently successful *in vitro* engineering of cartilage for all clinical needs.

Key words: *chondrocytes, chondrogenesis,* in vitro, *three-dimensional.*

This material is based on work supported by the following Institutes of the National Institutes of Health: the National Institute on Aging, Bethesda, MD 20892; the National Institute of Arthritis and Musculoskeletal and Skin Diseases, Bethesda, Maryland 20892-2350; and the National Institute of Dental & Craniofacial Research, Bethesda, MD 20892-2190.

Address all correspondence and requests for reprints to: Julie Glowacki, PhD, Orthopedic Research, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115.

INTRODUCTION

The Clinical Problems

Adult human cartilage shows poor capacity for repair and regeneration. Explanations for this include the limited potential for chondrocyte proliferation, the capacity of chondrocytes to become catabolic in response to pathological mediators, and the avascular nature of the tissue that prevents immigration of regenerative cells unless the lesion provides access to marrow. Degeneration of articular cartilage in diarthrodial joints is a hallmark of osteoarthritis and related disorders. Experimental studies show that superficial articular wounds usually incur selective loss of proteoglycan from the matrix, followed by inadequate attempts at cell proliferation and repair. Deeper defects that penetrate the subchondral bone and damage blood vessels and marrow integrity show a different response. Fibrocartilaginous repair tissue is formed, with functionally more suitable repair cartilage formed in smaller defects. Various surgical procedures have been developed for greater consist-

ency in repair (e.g., abrasion arthroplasty, microfracture, drilling, and transplantation of osteochondral plugs), but they are highly dependent upon technique and are limited to small lesions.

The motivation for tissue engineering is to promote biological repair or regeneration. The conceptual approaches for acquired or congenital deficiencies of cartilage include implantation of inert substitutes for discontinuities or missing parts, drug or matrix treatments to stimulate tissue regeneration, autogenous cell or tissue transfer, or *in vitro* production of tissues or tissue equivalents for implantation.

Among the orthopedic problems that may be considered for cartilage tissue engineering, articular cartilage lesions have shown the earliest applications for cell-based therapy. Other important needs are for replacement of damaged intervertebral discs and knee menisci. Craniomaxillofacial deficiencies, both congenital and acquired, may also be suitable for engineered cartilage. Congenital problems include structural deficiencies of cartilage in midface deformities, severe hemifacial microsomias, and microtia. Temporomandibular joint diseases can occur with severe damage in the joint disc/meniscus and articulating surfaces.

DISCUSSION

Source of Cells

Different biological preparations have been proposed for repair of cartilaginous defects (**Table 1**). Theoretically, cartilage tissue appears well suited for transplantation; it lacks a blood supply, is nourished by diffusion, and has a low cell-to-matrix ratio. There are sites for donor tissue, especially in pediatric patients. Transplanted autogenous cartilage has been used successfully for construction of ears in children with congenital microtia or atresia, with excellent long-term maintenance (1). Long-term results of osteochondral shell allograft resurfacing of knees indicate better function in unipolar than bipolar cases (2). Segments of cartilage, however, are less suitable for repair of articular surfaces or intracartilaginous defects where bonding to the tissue bed is important.

Table 1.
Sources of chondrogenic tissue.

Cartilage autograft
Banked cartilage allograft
Perichondrium
Periosteum
Freshly isolated or expanded chondrocytes
Bone marrow
Progenitors/precursors
Chondroinduced skin fibroblasts

Consideration of cauliflower ear inspired Skoog to examine the potential of the perichondrium to form new cartilage in a space or void created between the perichondrium and underlying cartilage (3,4). Experimental studies showed that isolated rabbit-ear perichondrocytes had the potential for chondrogenesis, but that autogenous transplantation of sheets of perichondrium to eburnated patellae resulted in inconsistent amounts and quality of neocartilage (5). Appearance of capillaries in fibrous areas suggested that focal angiogenesis is detrimental to cartilage repair and maintenance. Although there have been some reports on the clinical utility of perichondrial transplants (6), there is only limited donor material available in adults.

Periosteum is more plentiful than perichondrium in adults. Ham's classic studies in fracture healing pointed to the periosteum as the source of neochondrogenesis in callus tissue (7). Transplanted autogenous periosteum produces an admixture of new bone and cartilage, probably depending upon vascularity of the microenvironment in the recipient bed (8). Early attempts to resurface cartilaginous defects with transplanted periosteum gave variable success (9). More consistent results were obtained with continuous passive motion of joints repaired with periosteum (10).

Although progress in this area has been slow, there has been enthusiasm for the use of freshly isolated or expanded cells because of the good viability of transplanted chondrocytes (11). Defects created in articular cartilage that were filled with cultured chondrocytes showed significant repair tissue with features of normal articular cartilage (12). In that study, 80 percent of cartilage was reconstituted with the autologous chondrocytes, compared with only 18 percent in ungrafted controls. In carefully selected cases, the first clinical use of autogenous, expanded chondrocytes showed the potential for excellent-to-good results, especially in the femoral condyle (13). Thus, it is clear from experimental and clinical studies that adult chondrocytes can be expanded *in vitro* and used to produce cartilage *in vivo*.

There are, however, critical requirements for the successful expansion of chondrocytes *in vitro*. Moderate

seeding density and frequent subcultivations usually optimizes growth of most cell types. If that approach is used for chondrocytes, however, there may be a progressive, irreversible loss of function. Monolayer cultures of serially passaged chondrocytes fail to produce cartilage matrix; this phenomenon was described as the dedifferentiation of chondrocytes *in vitro* (14). It has been known for many decades that chondrogenesis can be enhanced if chondrocytes are seeded at high density (15), suspended in solution (16), isolated as chondrons (17), or cultured as pellets (14). Conditions that favor maintenance of phenotype are usually not those that favor increases in numbers (18). As a result, there may be limitations in the numbers of suitable cells that can be grown *in vitro* for subsequent repair of cartilage defects.

Bioreactors are commonly used for large-scale expansion of cell numbers, especially for cells that grow in suspension. Such bioreactors provide mixing to improve the rate of proliferation and yield of cells, but the fluid dynamics may not be appropriate for tissue morphogenesis. Scientists at NASA's Johnson Space Center developed low shear stress culture devices, called Rotating-Wall Vessels (RWVs), that support the accelerated proliferation and organization of cells into differentiated tissues or organoids (19). The unique conditions provided by RWVs stimulated chondrocytes to form large (5-mm) aggregates of cartilage in suspension (20).

As an alternative to harvesting normal cartilage as a source of chondrogenic cells, it is possible to differentiate chondrocytes from other cell types. Use of transgenes or multipotential stem cells has such possible applications if technical and other hurdles can be overcome. It is clear that subpopulations of marrow-derived cells (21) and muscle-derived satellite cells (22) have the capacity to give rise to chondrocytes. Whether chondrocytes that are differentiated *in vitro* from bone marrow preparations will maintain the articular phenotype or mature into hypertrophic chondrocytes may depend upon the site into which they are implanted or the mediators in their microenvironment (23). More information about the plasticity of chondrocytes and their potential for developing endochondral bone will be needed for clinical applications. It has also been reported that synovial tissue contains cells capable of forming cartilage in the presence of TGFβ1 (24).

In addition, human dermal fibroblasts have been shown to produce cartilage matrix chondroitin sulfate after culture with osteoinductive demineralized bone matrix (25). That monolayer system was found to be limited, however, because it did not optimize contact between the osteoinductive matrix and target cells. A three-dimensional (3-D) collagen/demineralized bone powder sponge system was developed that allowed for high-density culture with good nutrient exchange (26). Chondrogenesis was improved with a modification having a packing geometry of the demineralized bone powder that optimized cell/matrix interactions (27). On the basis of immunohistochemical, immunochemical (28), and molecular (29) assays, it was shown that this 3-D system supports the migration, viability, and chondroinduction of human dermal fibroblasts (**Figure 1**). These results suggest the possibility that fibroblasts from a patient's skin biopsy could be expanded into very large numbers *in vitro* with subsequent transdifferentiation into chondrocytes by exposure to demineralized bone or, theoretically, to putative differentiation factors or chondrogenic master transgenes. The resulting chondrocytes would thus be a source of autogenous cells for engineering cartilage tissue for that patient.

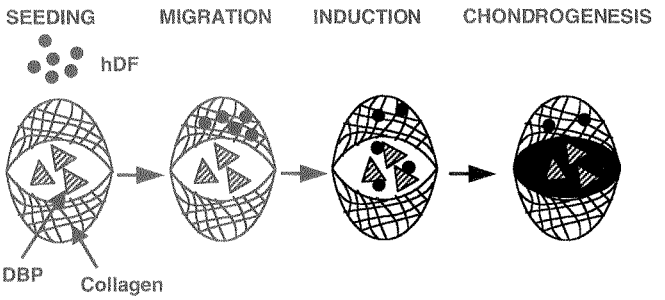

Figure 1.
Representation of the process of *in vitro* chondroinduction. Human dermal fibroblasts (hDF) are seeded on top of the composite sponge made of two layers of collagen around a packet of demineralized bone powder (DBP). The cells migrate through the collagen layer, attach to the particles of DBP, and are induced to produce extracellular cartilage matrix.

Carriers and Scaffolds

Delivery of simple cell suspensions is of limited value in musculoskeletal applications because of the requirement that the cells be retained at the desired site. Isolated chondrocytes lack adherence to the lesion sites and suspensions produce fibrocartilage or small foci of cartilage at best. Fluid carriers or 3-D scaffolds can be used for delivery and retention of cells. Popular natural hydrogels, such as alginates (30), fibrin (31), collagen gels, or admixtures (32,33), are useful to contain or immobilize cell suspensions. *In vitro* properties of alginate/fibrin beads have been enhanced with additives like

hyaluronan (34). Viscous suspensions of chondrocytes in hyaluronic acid preparations were used for cartilage regeneration in 3-year-old chickens (35).

In addition to their important function as a carrier of cells, 3-D scaffolds are useful to define the space for the new tissue, and, potentially, to enhance the maturation and function of the regenerated tissue. Candidate scaffolds or matrices include natural polymeric materials, synthetic polymers, biodegradable polymers, and polymers with adsorbed proteins or immobilized functional groups. Details of the manufacturing and properties of the synthetic materials have been reviewed (36).

Naturally occurring matrices or their components have advantages because of their outstanding biocompatibility properties. Devitalized cartilage matrix was used as a substrate for neochondrogenesis by isolated chondrocytes (37). The matrix/chondrocyte constructs were assembled with fibrin and shown to become mechanically integrated with the substrate (38). Other preparations of cartilage post-extraction remains have been used to support *in vitro* chondrogenesis (39).

Favored extracellular matrix (ECM) molecules of connective tissues are collagen, hyaluronan, and glycosaminoglycans. Porous, 3-D sponges comprised of collagen fibers support the viability of chondrocytes and their production of cartilage matrix *in vitro* (28). Others have used collagen gels for cultured chondrocytes, but, in contrast to scaffolds made of collagen fibers, those collagen gels have a tendency to contract in the presence of serum (40).

Synthetic polymers are used as 3-D scaffolds, or supports, for engineered cartilage. Synthetic polymers have theoretical advantages regarding plentiful supply, precise control of composition and material properties, and possibilities of biocompatible and resorbable features. Polymers comprise two major categories: those that are permanent and those that are temporary because they are resorbed by the body. Permanent polymers have been useful for many applications in orthopedics. Polyethylene in the ultra-high-molecular weight formulation is used for the articulating surface components in joint prostheses. Its clinical success is attributed to its low coefficient of friction and low rate of wear; however, longer use indicates significant generation of wear debris that contributes to implant loosening. Another important polymer is polymethylmethacrylate, used as a cement for fixation of implants to bone. Permanent scaffolds are not attractive for tissue engineering because they would interfere with tissue turnover and remodeling.

The key requirements of bioresorbable materials are that 1) their rates of degradation must be compatible with the intended use, and 2) the products of their degradation must be nontoxic. Of the synthetic materials, polyglycolic acid (PGA), polylactic acid (PLA), and their copolymers are most widely studied. They have been used clinically since their introduction as sutures in the 1970s. Their rate of resorption is considered short in comparison to other polymers like polycaprolactone (PCL), which has a longer half-life more suited to drug delivery applications. Matrices of PGA provided a template for new cartilage formation by chondrocytes (41). With precise control of culture conditions, bioreactors may provide improved growth and matrix synthesis (42,43).

Regulation of *In Vitro* Chondrogenesis

In addition to general aspects of good nutrient exchange and absence of conditions like turbulent flow (43), it is possible to modulate the quantity and quality of cartilage engineered *in vitro*. Serum factors and various growth factors have been shown to modulate cartilage matrix synthesis in 3-D cultures, especially fibronectin (44), insulin-like growth factor-I (IGF-I; 44,45), transforming growth factor β (TGF-β; 45), and platelet-derived growth factor (PDGF; 40).

Insoluble factors also modulate cell behavior in 3-D cultures. This understanding is founded on the appreciation among developmental biologists that ECM components influence the behavior of the cells that secrete them and on the subsequent discovery of cell membrane receptors for ECM molecules. The integrin receptors for protein motifs (or ligands) are the best studied, but another category of proteoglycan receptors includes CD44, which mediates chondrocyte binding to hyaluronan (46). Intra-articular injections of hyaluronan have been shown to have a protective effect in experimental osteoarthritis (47) and in clinical studies (49). This is of relevance to tissue engineering in that addition of hyaluronan to chondrocyte preparations appears to promote chondrogenesis (34,35). One of the earliest successes with chondrocyte therapy for repair of articular cartilage used a carrier for the cells that was a gel with ECM components produced by chondrocytes *in vitro* (49). It is notable that another ECM preparation, demineralized bone matrix, induces chondrocyte differentiation *in vivo* (50,51) and with human dermal fibroblasts *in vitro* (28,29).

Cartilage *in vivo* is well suited to adapt to the intermittent loads placed upon joints. It has been shown that mechanical compression modulates biosynthesis in carti-

lage slices (52,53) and in isolated chondrocytes (54). Preliminary results indicate that hydrostatic fluid pressure enhances chondrogenesis in 3-D culture (55).

Thus, it is evident that integrated optimization of constituents of the culture medium, components of the 3-D scaffold, and the mechanical environment should provide more consistent engineered tissue of desired quality to function where implanted.

Obstacles

Widespread discourse about the early experiments with tissue and organ engineering has generated public demand and expectations that engineered tissues will be available before long. There are, however, critical hurdles that need to be overcome. In the case of engineered cartilage, it would seem preferable to avoid harvesting of normal tissue and have a single operation for implantation of engineered tissue. Mature chondrocytes are exceptional in their ability to serve catabolic as well as anabolic functions. Control to inhibit the chondrolytic activities of chondrocytes would seem important for maintaining engineered tissues. Consideration of the limited proliferative and regenerative capacity of adult chondrocytes and the potential for dedifferentiation upon expansion also leads to the goal of an alternate source of cells. Use of xenogeneic or allogeneic cells requires selective shattering of the immunogenicity barrier in transplantation.

Only limited materials are currently available as carriers or scaffolds. Innovative synthetic materials, such as polypeptides or novel biodegradable polymers, need to be evaluated.

There remain difficulties in incorporation of neocartilage with adjacent healthy tissue. We have incomplete understanding of the relationship between cartilage and vascular response to wounding. Engineered cartilage needs to attach to the implantation site without evoking an angiogenic response. Enhancement of cartilage's antiangiogenic activity may be needed. If remodeling of the implant/tissue bed interface is required for integration, it must not proceed unregulated. These metabolic processes are not yet fully understood.

Future Directions

Current research is directed at solving each of these elements. Progress to date has been enabled by multidisciplinary attacks with creative solutions. Possible alternatives to the limited numbers of autogenous cells may include microencapsulation or modification of xenogeneic or allogeneic cells. It is conceivable that genetically modified cells could be grown on a biocompatible scaffold with internal signals for programmed histogenesis. Advances in materials design may generate "smart" scaffolds that will control tissue topology and have surface modifications to stimulate cell attachment, differentiation, and growth. Articular cartilage is a relatively simple tissue because of its cellular homogeneity and avascularity. Composite engineered tissues or organs, like an engineered joint, lie as a future goal. Cross-disciplinary interactions are likely to accelerate progress and to mediate application of advances made in other fields to consistently successful *in vitro* engineering of cartilage for all clinical needs.

REFERENCES

1. Brent B. Auricular repair with autogenous rib cartilage grafts: two decades of experience with 600 cases. Plast Reconstr Surg 1992;90:355–74.
2. Chu CR, Convery FR, Akeson WH, Meyers M, Amiel D. Articular cartilage transplantation. Clinical results in the knee. Clin Orthop 1999;360:159–68.
3. Ohlsen L, Skoog T, Sohn SA. The pathogenesis of cauliflower ear. An experimental study in rabbits. Scand J Plast Reconstr Surg 1975;9:34–41.
4. Engkvist O, Johansson SH, Ohlsen L, Skoog T. Reconstruction of articular cartilage using autologous perichondral grafts. A preliminary study. Scand J Plast Reconstr Surg 1975;9:203–9.
5. Upton J, Sohn SA, Glowacki J. Neocartilage derived from transplanted perichondrium: what is it? Plast Reconstr Surg 1981;68:166–72.
6. Brent B, Ott R. Perichondro-cutaneous graft. Plast Reconstr Surg 1978; 62:1–14.
7. Ham AW. A histological study of the early phases of bone repair. J Bone Joint Surg 1930;12:827–44.
8. Cohen J, Lacroix P. Bone and cartilage formation by periosteum: assay of experimental autogenous grafts. J Bone Joint Surg 1955;37A:717–30.
9. Ruback JM. Reconstruction of articular cartilage defects with free periosteal grafts: an experimental study. Acta Orthop Scand 1982;53:175–80.
10. O'Driscoll, Salter RB. The induction of neochondrogenesis in free intra-articular periosteal autografts under the influence of continuous passive motion. An experimental investigation in the rabbit. J Bone Joint Surg 1984;66:1248–57.
11. Green WT Jr. Articular cartilage repair: behavior of rabbit chondrocytes during tissue culture and subsequent allografting. Clin Orthop 1977;124:237–50.
12. Grande DA, Pitman MI, Peterson L, Menche D, Klein M. The repair of experimentally produced defects in rabbit articular cartilage by autologous chondrocyte transplantation. J Orthop Res 1989;7:208–18.

13. Brittberg M, Lindhal A, Nilsson A, Ohlsson, C, Isaksson O, Peterson L. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. N Engl J Med 1994;331:889–95.
14. Holtzer H, Abbott J, Lash J, Holtzer A. The loss of phenotypic traits by differentiated cells *in vitro* I. Dedifferentiation of cartilage cells. Proc Natl Acad Sci U S A 1960;46:1533–42.
15. Solursh M, Meier S. Effects of cell density on the expression of differentiation by chick embryo chondrocytes. J Exp Zool 1974;187:311–22.
16. Horwitz AL, Dorfman A. The growth of cartilage cells in soft agar and liquid suspension. J Cell Biol 1970;45:434–8.
17. Lee GM, Poole CA, Kelley SS, Chang J, Caterson B. Isolated chondrons: a viable alternative for studies of chondrocyte metabolism *in vitro*. Osteoarthritis Cartilage 1997;5:261–74.
18. Glowacki J, Trepman E, Folkman J. Cell shape and phenotypic expression in chondrocytes. Proc Soc Exp Biol Med 1983;172:93–8.
19. Goodwin TJ, Jessup JM, Wolf DA. Morphologic differentiation of colon carcinoma cell lines HT-29 and HT-29KM in rotating-wall vessels. In Vitro Cell Dev Biol Anim 1992;28:47–60.
20. Baker TL, Goodwin TJ. Three-dimensional culture of bovine chondrocytes in rotating-wall vessels. In Vitro Cell Dev Biol Anim 1997;33:358–65.
21. Wakitani S, Goto T, Pineda SJ, Young RG, Mansour JM, Caplan AI, et al. Mesenchymal cell-based repair of large, full-thickness defects of articular cartilage. J Bone Joint Surg Am 1994;76:579–92.
22. Young HE, Mancini ML, Wright RP, Smith JC, Black AC Jr, Reagan CR, et al. Mesenchymal stem cells reside within the connective tissues of many organs. Dev Dyn 1995;202:137–44.
23. Yoo JU, Barthel TS, Nishimura K, Solchaga L, Caplan AI, Goldberg VM, et al. The chondrogenic potential of human bone-marrow-derived mesenchymal progenitor cells. J Bone Joint Surg Am 1998;80:1745–57.
24. Nishimura K, Solchaga LA, Caplan AI, Yoo JU, Goldberg VM, Johnstone B. Chondroprogenitor cells of synovial tissue. Arthritis Rheum 1999;42:2613–7.
25. Glowacki J. Cellular reactions to bone-derived material. Clin Orthop 1996;324:47–54.
26. Mizuno S. Lycette C, Quinto C, Glowacki J. A collagen/DBP sponge system designed for *in vitro* analysis of chondroinduction. Mat Res Soc Symp Proc 1992;252:133–40.
27. Mizuno S, Glowacki J. A three-dimensional composite of demineralized bone powder and collagen for *in vitro* analysis of chondroinduction of human dermal fibroblasts. Biomaterials 1996;17:1819–25.
28. Mizuno S, Glowacki J. Chondroinduction of human dermal fibroblasts by demineralized bone in three-dimensional culture. Exp Cell Res 1996;227:89–97.
29. Glowacki J, Yates K, Little G, Mizuno S. Induced chondroblastic differentiation of human fibroblasts by three-dimensional culture with demineralized bone matrix. Mat Sci Eng C 1998;6:199–203.
30. Beekman B, Verzijl N, Bank RA, von der Mar K, TeKoppele JM. Synthesis of collagen by bovine chondrocytes cultured in alginate; posttranslational modifications and cell-matrix interaction. Exp Cell Res 1997;25:135–41.
31. Perka C, Spitzer RS, Lindenhayn K, Sittinger M, Schultz O. Matrix-mixed culture: new methodology for chondrocyte culture and preparation of cartilage transplants. J Biomed Mater Res 2000;49:305–11.
32. de Chalain T, Philips JH, Hinck A. Bioengineering of elastic cartilage with aggregated porcine and human auricular chondrocytes and hydrogels containing alginate, collagen, and kappa-elastin. J Biomed Mater Res 1999;44:280–8.
33. Scouten WH. Matrices and immobilization methods for cell adhesion/immobilization studies. Bioprocess Technol 1995;20:233–65.
34. Lindenhayn K, Perka C, Spitzer R, Heilmann H, Pommerening K, Mennicke J, et al. Retention of hyaluronic acid in alginate beads: aspects for *in vitro* cartilage engineering. J Biomed Mater Res 1999;44:149–55.
35. Robinson D, Halperin N, Nevo Z. Regenerating hyaline cartilage in articular defects of old chickens using implants of embryonal chick chondrocytes embedded in a new natural delivery substance. Calcif Tissue Int 1990;46:246–253.
36. Lanza RP, Langer R, Chick WL, editors. Principles of tissue engineering. Austin: R.G. Landes Company and Academic Press; 1997.
37. Peretti GM, Randolph MA, Caruso EM, Rossetti F, Zaleske DJ. Bonding of cartilaginous matrices with cultured chondrocytes: an experimental model. J Orthop Res 1998;16:89–95.
38. Peretti GM, Bonassar LJ, Caruso EM, Randolph MA, Trahan CA, Zaleske DJ. Biomechanical analysis of a chondrocyte-based repair model of articular cartilage. Tissue Eng 1999;5:317–26.
39. Nehrer S, Breinan HA, Ramappa A, Shortkroff S, Young G, Minas T, et al. Canine chondrocytes seeded in type I and type II collagen implants investigated *in vitro*. J Biomed Mater Res 1997;38:95–104. Published erratum appears in J Biomed Mater Res 1997;38:288.
40. Weiser L, Bhargava M, Attia E, Torzilli PA. Effect of serum and platelet-derived growth factor on chondrocytes grown in collagen gels. Tissue Eng 1999; 5:533–44.
41. Vacanti CA, Langer R, Schloo B, Vacanti JP. Synthetic polymers seeded with chondrocytes provide a template for new cartilage formation. Plast Reconstr Surg 1991;87:753–9.
42. Freed LE, Vunjak-Novakovic G, Langer R. Cultivation of cell-polymer cartilage implants in bioreactors. J Cell Biochem 1993;51:257–64.
43. Vunjak-Novakovic G, Martin I, Obradovic B, Treppo S, Grodzinsky AJ, Langer R, et al. Bioreactor cultivation conditions modulate the composition and mechanical properties of tissue-engineered cartilage. J Orthop Res 1999;17:130–8.
44. Martin JA, Buckwalter JA. Effects of fibronectin on articular cartilage chondrocytes proteoglycan synthesis and response to insulin-like growth factor–I. J Orthop Res 1998;16:752–7.
45. van Osch GJ, van den Berg WB, Hunziker EB, Hauselmann HJ. Differential effects of IGF-I and TGF beta-2 on the assembly of proteoglycans in pericellular and territorial matrix by cultured bovine articular chondrocytes. Osteoarthritis Cartilage 1998;6:187–95.
46. Ishida O, Tanaka Y, Morimoto I, Takigawa M, Eto S. Chondrocytes are regulated by cellular adhesion through CD44 and hyaluronic acid pathway. J Bone Miner Res 1997;12:1657–63.
47. Shimizu C, Yoshioka M, Coutts RD, Harwood FL, Kubo T, Hirasawa Y, et al. Long-term effects of hyaluronan on experimental osteoarthritis in the rabbit knee. Osteoarthritis Cartilage 1998;6:1–9.

48. Frizziero L, Govoni E, Bacchini P. Intra-articular hyaluronic acid in the treatment of osteoarthritis of the knee: clinical and morphological study. Clin Exp Rheumatol 1998;16:441–9.
49. Itay S, Abramovici A, Nevo Z. Use of cultured embryonal chick epiphyseal chondrocytes as grafts for defect in chick articular chondrocytes. Clin Orthop 1987;220:284–303.
50. Reddi AH, Huggins CB. Biochemical sequences in the transformation of normal fibroblasts in adolescent rats. Proc Natl Acad Sci U S A 1972;69:1601–5.
51. Mulliken JB, Glowacki J. Induced osteogenesis for repair and construction in the craniofacial region. Plastic Reconstr Surg 1980;65:553–9.
52. Kim YJ, Bonassar LJ, Grodzinsky AJ. The role of cartilage streaming potential, fluid flow and pressure in the stimulation of chondrocyte biosynthesis during dynamic compression. J Biomech 1995;28:1055–66.
53. Buschmann MD, Gluzband YA, Grodzinsky AJ, Hunziker EB. Mechanical compression modulates matrix biosynthesis in chondrocyte/agarose culture. J Cell Sci 1995;108:1497–508.
54. Smith RL, Rusk SF, Ellison BE, Wessells P, Tsuchiya K, Carter DR, et al. *In vitro* stimulation of articular chondrocyte mRNA and extracellular matrix synthesis by hydrostatic pressure. J Orthop Res 1996;14:53–60.
55. Mizuno S, Tateishi T, Ushida T, Ohshima N, Sledge CB, Glowacki J. Effects of soluble, insoluble, and mechanical factors on bovine chondrocytes in 3-dimensional culture. 43rd Annual Meeting, Orthopedic Research Society. San Francisco, CA; February 9–13, 1997.